

REMARKS

This response is due on April 30, 2004. A fee for a three-month extension of time is attached; no other fees are believed to be due; however, should any fees be properly due in connection with the filing of this document, or should the extension of time fee be inadvertently omitted, the Commissioner is hereby authorized to deduct any such fees from Marshall, Gerstein & Borun, LLP account number 13-2855.

I. Status of Claims

Claims 1-36 are pending in the application. Claims 24-36 were previously withdrawn as directed to non-elected subject matter, and claims 1-23 were examined in the last office action. Claims 24-36 are cancelled herein above. Claims 1-23 were rejected as allegedly being anticipated under 35 U.S.C. 102(b) and under 35 U.S.C. §103(a). Claims 2, 7, and 15 were objected to as being in improper format. Applicants have addressed the formalities with respect to claims 2, 7 and 15 and traverse the rejections of claims under 35 U.S.C. 102(b) and under 35 U.S.C. §103(a).

II. Formalities

Claim 2 was objected to for lacking the transitional word "wherein" in the claim, and has been amended in accordance with the Examiner's suggested amendment.

Claims 7 and 15 were objected to for being in improper multiple dependent format. Applicants believe that the above amendment to these two claims places them in proper format in accordance with MPEP §608.01(n).

In view of the above, Applicants request that the objects to the claims be withdrawn. Should the Examiner wish to discuss these amendment further, he is respectfully invited to contact the undersigned.

III. Art-based Rejections of the Claims

Claims 1 to 10 and 12 to 23 were rejected under 35 U.S.C. §102(b) as allegedly being anticipated by Zabeau et al. (EP 534 585 A1). Claim 11 was rejected under

35 U.S.C. §103(a) as allegedly being rendered obvious by a combination of Zabeau et al. (EP 534 585 A1) as applied to claims 1-10 and 12-23 and further in view of the teachings of Wang et al (Science 280:1077-1081, 1998) and further in view of Mead et al., (WO 94/21663). Applicants traverse these rejections and provide the following comments for the Examiner's consideration.

All of the rejections of the pending claims are based either wholly, or in some part, on the disclosure of EP 534 585 A1. Hence, Applicants believe that it would be germane to discuss the methods described in this European patent in further detail before proceeding with showing how this document fails to anticipate or render obvious the claimed subject matter of the instant application.

a. Disclosure of EP 534 585 A1

EP 534 585 A1, a document which has a common inventor (Zabeau) with the present application, is directed to a general method for DNA fingerprinting. In a general sense this method is an improvement over other methods of DNA fingerprinting making the method applicable to a complicated mixture of fragments in a DNA sample. In that method, a target DNA molecule is *initially* digested with one or more restriction endonuclease(s) (see e.g., EP 534 585 A1 description at page 3, line 23-25: "we have devised a new method to amplify . . . restriction fragments obtained after cleaving the DNA of an organism with at least one restriction enzyme.") This point, the **ends of the restriction fragments** are modified by adding oligonucleotide linkers or adapters to the ends (See EP 534 585 A1 page 3, line 26). By doing this, the method increases the length of the ends of the fragments that are "restriction endonuclease specific" that are usually only 2 to 8 nucleotides in length, and hence "too short to design primers for PCR amplification." (see EP 534 585 A1 page 3, line 28).

Thus, the linkers or adapters that are added onto the ends of the restriction fragments to "serve as a anchor base for the primers used in the PCR amplification" (see EP 534 585 A1 page 6, line 11). The adapters then "serve as templates for the primers . . . for subsequent PCR amplification" (see EP 534 585 A1 page 6, line 37-38). The methods disclosed in EP 534 585 A1 are used to "limit the number of restriction fragments which are

to be amplified. This is done by preselecting a subset of tagged restriction fragments so that only a small number of tagged restriction fragments will be amplified during the PCR amplification reaction." (see EP 534 585 A1 page 7, lines 11-14). In doing so, EP 534 585 A1 seeks to alleviate the problem in previous fingerprinting type methods, namely that "restriction endonuclease digests of genomic DNA . . . yields very large numbers of restriction fragments." (see EP 534 585 A1 page 6, lines 56-57).

In reviewing the entire disclosure of EP 534 585 A1, it is apparent that (1) the methods described in that document only uses one initial restriction enzyme digestion step to generate restriction endonuclease digests; and (2) the ends of those digests are specifically adapted using adapter or linker oligonucleotide sequences that facilitate subsequent PCR amplification. These two features are key to the methods of EP 534 585 A1, and also are key to how the methods described EP 534 585 A1 are readily distinguished from the methods of the present invention.

b. Subject Matter of Claims of the Present Invention

As indicated above, claims 1-23 are pending in the instant application. Of these claims, claim 1 describes the subject matter of the invention, which is readily distinguishable from that of the cited art. As all the remaining claims are dependent from or contain additional features in addition to the features of claim 1, the present section focuses on discussing the invention as claimed in claim 1.

Claim 1 describes a method of an endonuclease site polymorphism (ESP) in DNA, which involves an initial fragmentation step (b). The DNA fragments that are generated in step (b) are subjected to a **second** endonuclease reagent (step (c)). The products from the second endonuclease reaction are then amplified (step (d)) and analyzed "to determine which target fragments are amplified and/or which target fragments are not amplified; and wherein target DNA fragments which are amplified lack a recognition site for the probe restriction endonuclease reagent and target fragments having a recognition site for the probe restriction endonuclease reagent are not amplified." (step (e)).

Thus, the method uses a first fragmentation step (step b) in which a first one or more restriction endonuclease(s) serve as "sampling enzyme(s)" (see summary of the invention page 5, lines 23-26). This first fragmentation step is followed by a second fragmentation step (step (c)), in which a second endonuclease-based fragmentation step is employed that is preferably "a different restriction enzyme, the probing enzyme also referred to as a probe restriction endonuclease reagent." (page 5, lines 29-31). Thus, the present method uses a *combination of at least two separate restriction endonuclease-based fragmentation reactions*, to produce a "more accurate distinction between variable nucleotides than is possible" with a single "restriction enzyme-dependent assay that essentially detect fragment length polymorphism." (See page 12, line 10 through page 13, line 2).

Thus, the present invention relies on the use of two endonuclease steps, (1) the use of a first endonuclease reaction as the "sampling" reaction to generate DNA fragments for the restricted amplicon analysis and (2) a second endonuclease reaction as the "probing" reaction which determines the presence or absence of an endonuclease site polymorphism (ESP) that is recognized by the probing endonuclease. If the ESP is present, the DNA fragment will not become amplified in step (d), if the ESP is absent, the DNA will be amplified in step d. In this manner, the amplicon being amplified in step (d) is restricted.

With the above features for the restricted amplicon analysis of the present invention in mind, it is apparent that the methods of the invention are readily distinguishable from the cited prior art.

c. Claims 1-10 and 12-23 are novel over EP 534 585 A1

Claims 1-10, and 12-23 were rejected under 35 U.S.C. §102(b) as allegedly being anticipated by EP 534 585. Applicants respectfully traverse the rejection and request reconsideration in view of the present remarks.

It is an axiomatic that in order for a claim to be anticipated, a single item of prior art must disclose each element of the claim. See *Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 231 U.S.P.Q.2d 81, 90 (Fed. Cir. 1986). Applicants submit that the

disclosure of EP 534 585 simply fails to meet this criterion and, as such, does not anticipate any of the claims of the instant invention.

There are two independent claims under examination in the present application, i.e., claims 1 and 18, respectively. Each of these claims requires that a given target DNA being analyzed be subjected to a first fragmentation step (i.e., the fragmentation with the sampling restriction endonuclease carried out in step (b) in each of these claims.) In addition, each of these methods requires that a **second restriction endonuclease step be carried out** (i.e., the fragmentation step with the probing restriction endonuclease carried out in step (c)) in both of these claims). Applicants have reviewed the entire disclosure of EP 534 585 and that document neither explicitly or inherently contemplates a method that uses at least two separate endonuclease-based steps as required in the format of the present claims. As EP 534 581 fails to describe the use of a second probing restriction endonuclease or the use of ESPs that would be recognized by such an endonuclease in a method of performing a restricted amplicon analysis as described in the specification and detailed in claims 1 and 18, Applicants respectfully submit that EP 534 585 A1 fails to anticipate the methods of claims 1 and 18.

As the two separate endonuclease fragmentation reactions (i.e., a first endonuclease reaction to generate DNA fragments, followed by a second endonuclease reaction that recognizes ESPs) are an explicit feature of the independent claims of the invention, those reactions also are explicit features of each of the dependent claims. Regardless of the subject matter of the dependent claims e.g., specific endonucleases of claims 2-5 and 9-10, preparation of primers as in claim 6, ligation adapters of claims 7 and 15-16 and the like, the absence in EP 534 585 of the basic teaching of a **combination of at least two separate restriction endonuclease-based fragmentation reactions**, means that EP 534 585 A1 cannot anticipate any of the claims of the present invention.

In view of the above, Applicants respectfully request the rejection under 35 U.S.C. §102(b) based on EP 534 585 A1 be withdrawn and the claims be reconsidered for allowance.

d. Claim 13 is non-obvious over the combined teachings of EP 534 585 A1, Wang et al and Mead et al.

Claim 13 was rejected under 35 U.S.C. §103(a) as allegedly being rendered obvious by a combination of Zabeau et al. (EP 534 585 A1) as applied to claims 1-10 and 12-23 and further in view of the teachings of Wang et al (Science 280:1077-1081, 1998) and further in view of Mead et al., (WO 94/21663). Applicants traverse these rejections and provide the following comments for the Examiner's consideration.

In order to establish a *prima facie* case of obviousness the cited art must teach each element of the claimed invention. Moreover, where multiple references are used, there must be a suggestion or motivation to combine those references to arrive at the teachings of the invention and there must be a reasonable expectation of successfully realizing the invention in view of the teachings and the state of the art. *In re Vaeck*, 20 USPQ2d 1438, 1445 (Fed. Cir. 1991). These teachings must arise out of the prior art and not from the Applicants' own disclosure. As stated in MPEP 2143, these all three of criteria must be met in order to properly establish *prima facie* obviousness. It is the Applicants' position that none of these criteria are met by the teachings cited by the Examiner in the present case; a *prima facie* case of obviousness has not been established and the rejection should be withdrawn.

As discussed above, EP 534 585 fails to teach the basic methods of the present invention. EP 534 585 was admitted by the Examiner as not providing a teaching restriction endonucleases having recognition sequences of 2 nucleotides (see Office Action page 6) and thus was supplemented with Wang et al., (Science 280:1077-1081, 1998) which is cited as providing a teaching that a high percentage of single nucleotide polymorphisms occur within CpG dinucleotides and that analysis of CpG dinucleotides are advantageous for detection of polymorphisms. Mead et al., (WO 94/21663) was cited as teaching that digesting DNA with a probe restriction endonuclease reagent CGase I, which cleaves DNA at the dinucleotide CpG.

Regardless of the additional teachings of Wang et al. or Mead et al. Applicants submit that the obviousness enquiring over a combination of Zabeau et al., Wang et al., and/or Mead et al., cannot be initiated until the combination of all three references is shown

to provide a teaching of each of the elements of the claimed invention. As explained above, this requirement is not met by Zabeau alone. Therefore, in order for Wang et al., and/or Mead et al., to be properly combinable with Zabeau for the purposes of obviousness, these two references must first overcome the lack of teaching in Zabeau. As such, in addition to providing teachings of an endonuclease that has a recognition sequence of two nucleotides, these other two references must also provide a teaching of a method which uses a combination of two separate endonuclease fragmentations steps, followed by an amplification of those fragments and an analysis of the amplified products from the amplicon that was restricted by use of a combination of two separate endonuclease fragmentation reactions.

As there is no teaching of the basic methods of the independent claims of the invention in the combined disclosure, any combination remains flawed for the purposes of establishing obviousness of the dependent claims which necessarily incorporate all of the subject steps of the independent claims. As such, Applicants believe claim 13 is non-obvious over the combined disclosure because that disclosure fails to meet the first requirement of prima facie obviousness.

Even if one of skill in the art were to combine Zabeau with either Wang et al., or Mead et al., that combination would still only produce a method that uses a single restriction endonuclease step to generate DNA fragments that are cleaved at a recognition sequence of two nucleotides of CpG.

In view of the above comments, Applicants submit that claim 13 is non-obvious over the combined disclosure EP 534 585 with Wang et al., and/or Mead et al. and therefore, Applicants request that the rejection be withdrawn.

IV. Concluding Remarks

In summary, Applicants believe that the claims of the present invention are novel and non-obvious over EP 534 585 either alone or in combination with any of the other cited art. As Applicants have addressed the claim objections and shown the novelty and non-obviousness of the claimed subject matter, Applicants respectfully request that the rejections be withdrawn and the claims be favorably reconsidered for allowance. In the event that the

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Examiner wishes to discuss the above comments or claim language further, Applicants respectfully invite the Examiner to telephone the undersigned representative.

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Respectfully submitted,

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